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(54) Title: PROCESS FOR INTENSIFICATION OF FERMENTATIONS

(57) Abstract

The invention relates to a new process for intensification of fermentations, especially anaerobic, mesophilic, methane producing microbial fermentations under septic conditions, particularly sewage treatment, inoculum formation or increasing the digesting capacity of anaerobic sewage sludge containing mixed acetogenic-methanogenic or only methanogenic micropopulations. The process of the invention can be applied primarily in environmental control, particularly in sewage treatment, but it can also be used in other areas such as B12 coenzyme (cobamide coenzyme) fermentation and other fermentations performed preferably with a mixed, anaerobic, mesophilic methane producing micropopulation at septic conditions. In the process of the invention the system is submitted to cyclic electric stimulation. Preferred parameters of electric stimulation: square impulse type; amplitude, 30-250 V; frequency, 5-50 Hz; refractory time, 5-50 sec. Biogas production of the electrically stimulated process is about 70-80 % higher than that of the unstimulated one.

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PROCESS FOR INTENSIFICATION OF FERMENTATIONS

The invention relates to a new process for intensification of fermentations by applying electric stimulation in any stage(s) of the process.

The electric stimulation can be preferably applied for anaerobic, mesophilic, methane producing microbial fermentations under septic conditions, particularly for sewage treatment, inoculum formation or increasing the digesting capacity of anaerobic sewage sludge containing mixed acetogenic-methanogenic or only methanogenic micropopulations.

The process of the invention can be applied primarily in environmental control, particularly in sewage treatment, but it can also be used in other areas such as B12 coenzyme (cobamide coenzyme) fermentation and other fermentations performed preferably with a mixed, anaerobic, mesophilic methane producing micropopulation at septic conditions.

The worldwide deterioration of the natural environment has taken catastrophic proportions in the past two to three decades. The large scale development of both industry and agriculture, the rate of population increase all resulted in the disruption of the ecological balance. The atmosphere, hydrosphere and lithosphere are equally endangered by human activity detrimental to the environment.

Sewage treatment represents one of the major tasks of environmental control. In the first half of the century, according to the widely applied sewage treatment process (sewage originating mainly from municipal sources), sewage was aerated in large, open pools, then the activated sludge formed was submitted to fermenta-

tion in large, heated containers (digesters) at anaerobic conditions, at the simultaneous production of biogas, the sludge obtained as end product was left to desiccate on a sand bed, and finally utilized as organic fertilizer in agriculture.

During the decades following the Second World War the amount of sewage recirculated in the environment increased due to the enhanced water consumption of the rapidly developing industry and agriculture. The small number of low-capacity sewage treatment plants, working according to outdated technologies, could no longer process the increased loading thus industrial, agricultural and municipal sewage became the largest hazards endangering natural water resources.

By the 60s experts started recognizing that a.) the production structure, developed in the past decades, was wasteful as regards materials, resulting in the formation of excess masses of industrial and agricultural waste, thus, as a first stage, they began reducing sewage to a minimum by optimizing the large scale industrial processes, furthermore, b.) that the environmental projects can only be realized if suitable scientific research and development bases with an industrial background are established which requires a new industrial branch, new processes, the manufacturing of new equipments, tools, machines, instruments, etc.

Thereafter, as a result of the threatening increase of water requirements, and, not last but least the rapid increase in energy prices, extensive research and development projects were started to establish the composition as well as the microbial-biochemical properties of sewages.

By the second half of the 70s the development of economic, high capacity, anaerobic sewage treatment processes was initiated which by the 80s were introduced

in industrial scale [Anaerob Digestion (1988), Eds. E. R. Hall and P. N. Hobson, Pergamon Press, Oxford, Tokyo, Toronto, etc.] with the primary aim to recirculate the purified water resulting from the water treatment process either into the natural source or to the technology and to obtain significant amounts of energy in the form of methane converted from the organic material.

In the natural environment complex organic materials are digested by a mixed population of microorganisms. The microorganisms participating in the process are living in symbiosis, the product of one degradation step is the substrate for the activating group of the next step. At equilibrium conditions the process is stable and has high flexibility, i. e. the proportion of each group of microorganisms - within certain limits - flexibly adapts to the concentration of metabolites. In the anaerobic process producing biogas four distinct stages can be differentiated:

In the first stage the activity of hydrolizing microorganisms is the dominant feature (proteases, cellulase, etc.). Acting through extracellular enzymes high molecular mass, mostly insoluble polysaccharides, proteins and lipides are solubilized and digested at facultatively anaerobic conditions.

In the second stage metabolites of the first stage (carbohydrates, peptides, higher alkyl fatty acids, etc.) are further degraded by acidogenic microorganisms at facultatively anaerobic conditions, resulting in the formation of lower alkyl fatty acids, alcohols, hydrogen and carbon dioxide.

In the third stage mostly acetate is produced from the various organic acids and alcohols by the acetogenic microorganisms.

In the final stage methane is produced. Acetate and the C1 compounds (methanol, formic acid, methyl-

amines), as well as carbon dioxide and hydrogen serve as substrates of the methanogenic microoganisms for the conversion to methane. Known are also hydrogenophilic methanogens producing methane by utilizing carbon dioxide and hydrogen, furthermore acetotrophic methanogens producing methane and carbon dioxide by splitting acetate molecules.

In the first two stages the facultatively anaerobic microorganisms exert their function in the pH range of 4.5 to 5.5 while in the final two stages the obligatory anaerobic acetogens - methanogens in the pH range of 7 to 7.5 [Anaerob Digestion. Intern. Symp. in Cardiff (Wales, 1979); in Travemünde, FRG (1981); in Boston, USA (1983); in Guangzhou, China (1985); in Bologna, Italy (1988)]. In the natural environment the complex anaerobic digestion occurs at the same site, no optimal physicochemical conditions are ensured for individual bacterium groups consequently the output of the natural process is always lower than optimal.

In aerobic sewage treatment processes the air required for the microbial oxidation of the organic matter has to be mixed to the sewage. In the course of this procedure about 50 % of the organic carbon content is oxidized to carbon dioxide and the residual carbon becomes part of the activated sludge.

In the course of anaerobic sewage treatment procedures about 80 % of the digestable carbon is released as biogas in the form of methane + carbon dioxide. Furthermore, the anaerobic process is also more advantageous energetically, 1 kg of COD (chemical oxygen demand) is required for the formation of 0.5 cu.m. of biogas having a methane content of 55 - 60 % (25.5 MJ/Ncu.m.=6000 Kcal/Ncu.m.) which is equivalent to 0.3 kg of fuel oil (42 MJ/kg=10,000 Kcal/kg), i. e. it is an energy producing process, whereas 1 kg of COD in the

aerobic process requires 0.5 - 1 kWh energy during aeration.

Considering the more advangateous energetic conditions as well as the considerably lower quantity of sludge formed in the anaerobic process, which does not even require any final treatment and may be utilized for the amelioration of soils, attention was focused on the anaerobic procedures. Two parallel series of international symposia were initiated - and are continued uninterrupted ever since in intervals of 2 - 3 years - "ANAEROBIC DIGESTION" (1979, 1981, 1983, 1985, 1988 and 1990) and "MICROBIAL GROWTH ON C1 COMPOUNDS" (the 6th Conference was organized in 1989 in Göttingen).

The development of anaerobic processes producing biogas was initiated in the various countries of the world at different times, volumes and capacities, depending on the level of development and the specific problems posed (energy shortage, environmental control).

Two large groups can be differentiated if the volume, application and technical implementation of the processes are considered:

a.) Small scale biogas production. Converting the liquid manure, agricultural, communal and fecal wastes of individual households, farms and small communities in a common equipment to biogas. These equipments are called farm-biogas plants. The Sechuan-type biogas plant is a typical member of this group. In such a large country with scattered settlements the establishing of an energy network would have been expensive and slow, thus local energy sources were looked for. In 1985 there were already 4.2 million Sechuan-type farm-biogas plants operating in China. The situation is similar in other countries of the Third World, especially in India (Desai and Patel type plants). In the developed industrial-agricultural

countries - e. g. Netherlands, Belgium, Denmark, Germany -farm biogas plants started to be introduced by the end of the 60s and the 70s but later on the more economic industrial scale plants were developed.

b.) Industrial scale biogas production. There are two large groups of raw materials applied for biogas production: organic wastes of high dry material content [higher than 15 % (v/v)] (corn-blade, corn-stalk, wheat-straw, coir, etc.) and wastes of low or medium dry material content [lower than 15 % (v/v)]. The latter wastes are generated in large amounts in the industry, food-industry, agriculture and settlements in the form of municipal sewage.

Extensive research pursued for years has revealed that an up to date anaerobic process can be developed by increasing the concentration of the active methanogenic biomass, improving intrinsic contact and enhancing contact surfaces. Exploiting the well known aptitude of methanogens for forming conglomerates the retention time of the active biomass in the reactor was extended either by forming - using suitable techniques - a living aggregate in the biomass, i. e. granulates or forming an immobilized biofilm on an inert carrier. This also required designing of new reactors with significantly lower hydraulic retention time (HRT) - because of the substantially increased active surface - operating at higher dilution rate and as a consequence, having significantly lower volumes.

Furthermore it was also recognized that the output can be significantly increased if the complex anaerobic process is split up into two sterically separated stages -acidogenic and methanogenic - each ensuring optimal conditions for the respective microorganism group, and the sewage is led first into the acidogenic and then into the methanogenic unit.

The process to be developed depends primarily on the source, periodicity of appearance, and quality of sewage. Most sewage treatment plants are of individual design despite being very similar. The design should consider the following main features:

- The sewage can contain significant amounts of solids of eventually large particle size which without pretreatment (filtering sedimentation, etc.) can clog certain reactor types;

- The treated effluent of sewage with high dissolved organic material content (COD 40 - 50 kg/cu.m.) may contain, even at a conversion rate of 95 %, 2 - 2.5 kg of COD/cu.m. which should be reduced to the specified low COD value by further treatment (second anaerobic stage or aerobic treatment).

- Sewage of the chemical or pharmaceutical industry contains poorly digestable organic components (halogenated aliphatic, aromatic compounds, etc.) which may require eventual pretreatment or longer time for adaptation. Methanogenic microorganisms (methanogens) are reported in detail in the Progress in Industrial Microbiology [Vol. 16, pp. 233 - 237 (1982)], their morphology and physical properties by W. E. Balch, G. E. Fox et al. [Microbial Rev. Vol. 43, 260 - 296 (1979)] and their metabolism in the Handbook on Anaerobic Fermentations pp. 537 - 545 (1988), Eds.: Larry E. Erickson and Daniel Yee-Chak Fung. Marcel Dekker, Inc. New York, Basel].

The viability of the acetogenic-methanogenic micropopulation [Anaerob Digestion: Proceedings of the First Intern. Symposion on Anaerob Digestion. Eds.: D. A. Stafford, B. I. Wheatley, D. E. Hughes. pp. 15 - 20. Pergamon Press, Cardiff, Wales (1979)] is ensured by the reaction wherein acetate, hydrogen and carbon dioxide, produced by the acetogens, is continuously reduced by

the methanogens. The use of the mixed acetogenic-methanogenic micropopulation is the method of choice for the treatment of sewage containing medium length alkyl fatty acids, other organic acids, alcohols and organic amines [Handbook on Anaerob Fermentations, pp. 597 - 600 (1982). Eds. Larry E. Erickson, Daniel Vee-Chak Fung. Marcell Dekker, Inc. New York, Basel].

Inoculum, based on the freshly digested sewage sludge taken from the anaerobic second digester of municipal sewage treatment plants, was mainly applied for the production of the B12 coenzyme.

Stimulation is a widely applied tool in biology. Stimulus is an external effect exerted on the living organism which induces however distinct response reactions. Mechanical, heat, light, various chemical effects, but also electric current and radioactive irradiation all represent some type of stimulus. The artificial stimulation of cells is usually performed by an electric impulse as the energy of the stimulus can be easily regulated by varying its amplitude and duration. Due to this exact regulatory potential the electric current is the stimulation method of choice in scientific research. too [F. Brunó Straub: Biological Lexikon, Vol.2. pp. 280 - 281. Akadémiai Kiadó, Budapest, Hungary (1978)].

According to the U. S. Patent Specification No. 4,822,470 placing cells pretreated with continuous electric fields generated by 100 - 800 V/cm alternating current into an oscillating high intensity electric field of high radiofrequency (5 - 20 KV/cm and 50 KHz - 500 MHz) the cell membrane permeability is increased, transitorily small and large pores are formed on the cell membrane (electroporation) which may even induce cell fusion.

The method may be applied with high yields for

introducing biologically active compounds - DNA, RNA, proteins, antibodies, hormones, enzymes, growth factors, etc. - into various animal, human, plant and bacterium cells, as well as for the intercellular exchange thereof and for creating hybride cells with new biological properties through cell fusion.

The East German Patent Specification No. 248,140 (Wirtschaftspatent; economic patent) describes an aerobic, sterile fermentation performed in a fermentor placed in a direct current field. The fermentation broth, surrounded by two Helmholtz coils, is stimulated for 5 msec with intervals of 62 msec. The yield of the antibiotic fermentation is increased by about 10 % in this way and the process has the further advantage that no electrodes have to be immersed into the broth.

It is known that about 30 years ago B12 coenzyme was produced by a fermentation process from nutrients and microorganisms present in the sewage sludge, which, if desired, were supplemented with various further nutrients. The process had the high advantage that the fermentation was performed at nonsterile conditions, but had also the disadvantage that large amounts of sewage sludge had to be transported from the sewage treatment plant to the site of the fermentor, both the composition and the bacterium population of the sewage sludge were variable and there was also an opportunity for the growth of "wild strains" which inhibited the development of a stable bacterium population.

The Hungarian Patent Specification No. 153,740 describes a new process for the anaerobic, nonsterile production of the B12 coenzyme wherein the sewage sludge is added a single time to the culture medium containing the necessary nutrients, thereafter, following at least 5 inoculations, a mixed micropopulation is generated

which is self sufficient in taking over the role of the inoculum, producing fermentation broths with about 6 - 6.2 mg/l of B12 coenzyme content. However, this "sewage sludge free" procedure requires at least 5 inoculations, i. e. the adaptation of the microorganisms in the sewage sludge to B12 coenzyme production is proceeding rather slowly, the production level of the B12 coenzyme is low, requires many types of nutrients, i. e. the process is expensive.

Several further processes are known for producing the B12 coenzyme and for improving their production yields (U. S. Patent Specification Nos. 3,954,971 and 3,979,259) which attempt to intensify the above "sewage sludge free" procedure.

The Hungarian Patent Specification No. 167,658 was the first document describing a mesophilic mixed methane producer micropopulation containing Corynebacterium sp. 24A1, Corynebacterium sp. 62B9, Lactobacillus sp. 244 B/C1 and Propionibacterium sp. 239A1/6, deposited at the National Institute of Hygiene, Budapest, Hungary (OKI) under Nos. 00076, 00077, 00078 and 00079, resp.

However, this anaerobic, mesophilic, mixed, methane producer micropopulation was only slowly adaptable to media containing nutrients other than the usual ones, required 6-7 inoculations, and the duration of each passage cycle amounted to about 7 days, i. e. to obtain the above new mixed micropopulation adapted to large scale industrial production required about 40 - 50 days, which was time consuming and very expensive.

In the process described in the U. S. Patent Specification No. 4,752,584 (Hungarian Patent Specification No. 188,954, Hungarian Priority 16.09.1983) the inventors returned to the original sewage sludge and the micropopulation present there was directly adapted to a new nutrient medium, thus a new, anaerobic, mesophilic,

mixed, methane producer micropopulation was developed, and not the already developed anaerobic, mesophilic, mixed, methane producer micropopulation was adapted to a new nutrient medium.

However, on a specific medium any listed inoculum is suitable solely for the production of the B12 coenzyme.

Specification of technical data used in anaerobic sewage treatment processes:

Chemical Oxygen demand (COD) loading:
Dimension: COD g(kg)/l(cu.m.)fermentor/hour (day) [COD (g, kg) input per working volume of digester (or fermentor) (l, cu.m.) as a function of time (hour, day)].

Working volume: volume of digester (or fermentor) filled up with medium.

Hydraulic loading:
Dimension:
sewage influx l(cu.m.)/l(cu.m.) fermentor/day [sewage volume influx per working volume of digester (or fermentor) as a function of time]

Capacity of equipment:
Dimension:
converted COD g(kg)/l(cu.m.) fermentor/day [amount of COD converted to biogas per working volume of digester (or fermentor) as a function of time].

Rate of COD conversion (%): COD of effluent sewage g/l) is multiplied by effluent volume per time (l) and divided by the COD of sewage influx (g/l) multiplied by influx volume per time:

$$\% = \frac{\text{sewage efflux (l)} \times \text{effluent COD (g/l)}}{\text{sewage influx (l)} \times \text{COD input (g/l)}} \times 100$$

Hydraulic retention time (HRT):
Dimension: hour or day
Sewage retention time in the digester (or fermentor).
The value of HRT is reciprocal to the dilution rate (D).

Dilution rate (D): Volume of sewage flowing through the working volume of the digester (fermentor) in a time unit per working volume of the fermentor:

$$D = \frac{\text{sewage flow (l/day)}}{\text{working volume of fermentor (l)}}$$

Dimension: h^{-1} or day^{-1}

Methanogenic activity: ml of methane produced by 1 g of dry biomass in 1 day in the digester (or fermentor). Dimension: l biogas/l fermentor/day

Biogas production: Volume of biogas produced in 1 unit of the digester (or fermentor) working volume in a time unit.
Dimension: l biogas/l fermentor/day.

It is the objective of the present invention to update the usual biogas producing anaerobic sewage

treatment processes and anaerobic, nonsterile B12 coenzyme producing fermentations as well as to convert the methanol component of the B12 fermentation to fuel-methane, furthermore to improve significantly both the rate and capacity of sewage treatment processes and B12 coenzyme production.

In the course of our experiments it was found that

1.) adding anaerobic, digested sludge (e.g. produced according to Hungarian Patent Application No. 92 03241), activated by electric stimulation, to sewages obtained from various sources, a mixed microorganism population of high acetogenic-methanogenic activity is developed within a significantly shorter period of time than before thereby significantly accelerating the conversion of organic matter to biogas, or

2.) applying electric stimulation to an anaerobic, mesophilic methane and B12 coenzyme producing fermentation the activity of methanolysis is enhanced, resulting in the increase of B12 coenzyme production capacity, and,

3.) either of the processes can be realized in any type of reactor.

Based on the fact, that the electric stimulation resulted the same unexpected positive effect for different type of micropopulations, it should be regarded to be supported that the electric stimulation of fermentation medium (broth) can be applied for intensification of different type of fermentations.

According to an embodiment of the present invention if the stimulation is performed by direct current, low frequency, square impulses in a given frequency and amplitude range, and the short stimulation - interval periods are cyclically repeated during

several hours, the following changes can be recorded in the methanogenic process:

a.) The specific methanogenic activity of the culture (CH_4 ml/l_x broth/day) is increased by 50 - 80 % whereas the dissolved organic matter of the fermentation broth is reduced;

b.) during a stimulation period of some days microorganism aggregates of a particle size of 0.5 - 1.5 mm are formed in the samples investigated and the specific methanogenic activity of the aggregates is 4-5fold compared to the usual ones,

c.) even 70 - 80 days after discontinued stimulation the increased methanogenic activity remains stable, no significant decrease is detectable.

Based on our experiences a process was developed for preparing an activated inoculum by the electric stimulation of anaerobic digested sludge which, adapted to various sewage types of the food industry, is suitable to improve the capacity of anaerobic sewage treatment processes by 40 - 60 %. If desired, the electric stimulation can be repeated periodically during either the adaptation or the sewage treatment process or in the stationer (producing) stage of the fermentation.

The invention relates for a process for intensification of fermentations, which comprises applying electric stimulation of the fermentation medium in any stage or stages of the fermentation, i.e. in the inoculum preparing stage and/or in the adaptation stage and/or in the stacioner (producing) stage of the fermentation.

The invention can be exploited very advantageously for anaerobic, mesophilic, methane-producing

fermentations.

A preferred embodiment of the invention is an anaerobic, mesophilic, methane-producing fermentation for improving preferably sewage treatment procedures pursued under septic conditions by acetogenic-methanogenic micropopulations, which comprises

a) loading an air-tight fermentor, equipped with two electrodes, with a sewage sludge freshly obtained from the final digester of a municipal sewage treatment plant, which is preferably pretreated with cyclic electric stimulation, and maintaining an ascending, recirculating flow in the mesophilic temperature range for 1 - 4 days (the mixed micropopulation is adapted to the new environment, i.e. to the new equipment by this step), then

b) adding daily at least equal volumes of 0.1 - 3.5 g COD/litre sewage to the inoculum formed, starting cyclic electric stimulation, maintaining an intense ascending flow of the system, transferring the biogas formed to a gasometer and collecting the overflow which has equal volume with that of the sewage influx, thereafter

c) the COD loading of the sewage to be treated is gradually increased to a concentration of 3 - 40 g COD/litre, simultaneously the cyclic electric stimulation is maintained for 5 - 30 days, during which period a biomass of high methanogenic activity, adapted to the particular sewage, is developed, thereafter the electric stimulation is stopped and the sewage treatment is continued, if desired,

d) the treated sewage is discarded or purified further, the biogas formed is transferred into a gasometer and utilized, or,

The invention refers furthermore to an anaerobic, mesophilic, methane-producing fermentation

process preferably for improving B12 coenzyme production under septic conditions, applying a methanogenic micropopulation in a medium containing known nutrients which comprises submitting the fermentation broth to cyclic electric stimulation.

The invention refers furthermore to a process for preparing a new inoculum containing an anaerobic, mesophilic, mainly acetogenic-methanogenic, mixed micro-population by submitting the anaerobic digested sewage sludge to cyclic electric stimulation. A preferred embodiment of this process comprises the following steps:

a) 25 - 35 % (v/v) of an inoculum and 65 - 75 % (v/v) of a 1 - 4 g COD/litre sewage (calculated for working volume) are added to a batch fermentor, the system is homogenized, and the pH is adjusted to 6.5 - 7.5, then

b) batch fermentation is pursued in the mesophilic temperature range, under anaerobic conditions for 5 - 10, preferably for 6 - 8 days, then,

c) after 5 - 10, preferably 6 - 8 days, and following homogenization, 65 - 75 % (v/v) of the fermentation broth is removed and an equal volume of sewage, having a concentration of 1 - 4 g COD/litre, is added and the batch fermentation is repeated according to the procedure specified in paragraph a2.),

d) the batch fermentation is followed for further 5 - 12, preferably 6 - 10 day among similar conditions but the fermentation broth is electrically stimulated,

e) the obtained electrically stimulated inoculum is collected.

The electric stimulus is generated by direct current square impulses, having an amplitude of 30 - 250 V, a frequency of 5 - 50 Hz and a refractory time of 5 - 50 sec between impulses.

The artificial stimulation of cells, neurocytes and muscles is usually performed by electric impulses as the energy of the stimulation can be easily regulated by varying its parameters (amplitude, duration) [F. Brunó Straub: Biological Lexikon, Vol. 2. pp. 280 - 282 (1978), Akadémiai Kiadó, Budapest, Hungary].

Excitability and impulse conductivity are properties of all viable cells. In all cells the initiation and progress of the stimulatory process ia related to the variations of the electric properties in the cell membrane [H. Schaefer: Elektrobiologie des Stoffwechsels. Handbuch d. allgem. Pathologie, Band 4, Teil II. Berlin-Göttingen-Heidelberg, Springer (1957)].

Charges are separated by the external and internal surface of the cell membrane, thus at rest a potential difference is developed, the cell membrane is in a polarized state [A. L. Hodgkin., B. Katz: J. Physiol <u>108</u>, 37 (1949)].

Upon stimulation the polarized state is depolarized, and an action potential is created. At rest the potential difference between the external and internal surface of the cell membrane amounts to -90 mV, upon stimulation not only is this potential difference levelled off but a reverse polarization develops, amounting to +50 mV [P. Bálint: Physiology. pp. 38, 39, 59, 67. Medicina Press. Budapest, Hungary (1963)].

Course of action potential: for the creation of an action potential the stimulus has to attain a specific threshold level. The amplitude of the created stimulus remains stable at the experimental conditions specified.

No action potential is created at subminimal threshold levels nor is response induced by a supramaximal stimulus, only after a longer interval. For the re-

turn of initial excitability there must be an interval between two stimuli, this is the refractory time: interval between two stimuli [J. W. Woodbury, H. D. Patton: Medical Physiology and Biophysics (1960), Eds. T. C. Ruch, J. F. Fulton, 18th Ed. Saunders, Philadelphia-London].

No stimulus or action potential is detectable during current flow. Only the opening and closing of the circuit, and the changes in the stimulatory energy can be considered as stimulus. The action potential appears only after stimulation, as a result of it [P. Bálint: Physiology, p. 64 (1963), Medicina Press, Budapest, Hungary].

The rate of increase and decrease of the appearance and disappearance of a stimulus is of utmost importance this is why it is recommended to use the square impulse where the rate of stimulus increase is the highest due to the steepest signal shape.

Definitions of impulse techniques are the following [K. Tarnay: Electrone tube connections. 2nd Ed. Technical Press, Budapest, Hungary (1962)]:

Impulse:	Single voltage surge, single energy surge
Impulse shape:	Voltage time course
Impulse amplitude:	Voltage value (V)
Time of repetition:	Period between two impulses
Repetition frequency:	Number of stimuli in time unit
Impulse width:	Period during which voltage is at a stable level other than zero
Filling factor:	Impulse width per repetition time
Refractory time:	Time between two stimuli
Time of stimulation:	Duration of stimulation in the culture.

Specification of the electric stimulus applied in the process of the present invention:
Square impulse; amplitude 30 - 250 V, preferably 80 - 100 V; Frequency 5 - 50 Hz, preferably 20 - 30 Hz; refractory time 5 - 50 sec, preferably 10 - 20 sec, duration 4 - 12 h/day, preferably 5 - 8 h/day.

According to an embodiment of the present invention the inoculum is prepared by removing freshly digested sewage sludge from the digester of any municipal sewage treatment plant. These digesters have usually volumes of several thousands of cu.m., for industrial scale inoculum preparation some (1 - 2) cu.m. are required while some litres are sufficient for laboratory experiments. The freshly digested sewage sludge is usually transferred into a fermentor equipped with stirrer and heating jacket. In the laboratory a vessel with an air-tight cover is sufficient if mechanical stirring for the homogenization of the digested sludge is ensured.

According to the embodiment of the present invention the mesophilic temperature range required for the proliferation of microorganisms is of highest importance, this should be 25 - 40°C, preferably 30 - 38°C. The specified temperature range can be realized by any of the known methods, e. g. by circulating water or an other liquid of the required temperature in the fermentor jacket. In large fermentors no jacket is required as the fermentation is a heat generating process. In the laboratory the fermentor is placed either in an air-conditioned room or thermostate.

The industrial or laboratory fermentor is connected to a gasometer to ensure anaerobic conditions and to collect the biogas formed for further utilization. In laboratory experiments the measuring of the biogas volume is important for evaluating the experiment

as the specific biogas formation (ml biogas/l fermentation broth) is one of the major parameters of the process.

The freshly digested sewage sludge is adapted to the new conditions during 2 - 10, preferably 4 - 7 days, since a live system, a mixture of microorganisms has to be adapted to the new environment. Adaptation can also be performed by removing daily 5 - 10 % (v/v) of the sewage sludge and replacing it with an equal volume of digested sludge or some other sewage ready for purification.

The new equilibrium becomes stabilized within days then adaptation is discontinued and the electric stimulation of the system with a generator is started. The duration of the stimulation is 4 - 10, preferably 6 - 8 hours daily, continued for 5 - 12, preferably 6 - 10 days. Finally the new inoculum, containing the anaerobic, mesophilic, mostly acetogenic-methanogenic, mixed micropopulation is obtained which is required for the initiation of the treatment of sewages of various sources.

Sewage treatment can be improved by adding to the inoculum, submitted to electric stimulation described above, under nonsterile conditions, preferably daily, equal volumes of sewage, having a concentration of 0.1 - 3.5 g COD/l, if desired, in diluted form. According to an other variant of the process no electrically stimulated inoculum is used, but the sewage, having a concentration of 0.1 - 3.5 g COD/l and which is diluted, if desired, is directly added to the freshly digested sewage sludge of the municipal sewage treatment plant. Even in this case adapting the sludge for a couple of days to the environment is preferred. The amount and the rate of eventual dilution of the sewage added to the inoculum depends on the source and quality

of the sewage. The sewage to be treated has frequently concentrations higher than 0.1 - 3.5 g COD/l, then diluting is preferred, as thus the anerobic, mesophilic, mostly acetogenic-methanogenic, mixed micropopulation of the inoculum is faster adapted to the respective sewage. For the dilution water may be used, but the use of the effluent of the sewage treatment plant is the preferred diluent, because, as described in Example 3 of our patent specification covering sewage purification, the COD concentration of the sewage influx is 30 g/l and after a conversion of 92 % the effluent still has a COD concentration of 2.4 g/l which requires further purification. In our Example 4 the sewage of the dairy plant requires no dilution as - unlike the former one - its COD content is in the range of 0.1 - 3.5 g COD/l and in addition contains impurities which are easily converted to biogas. The duration of adaptation as well as the rate and necessity of diluting are always depending on the quality of the sewage to be purified, however, this requires no specific knowledge beyond that of the present invention and that known in the art.

Cyclic (regularly resumed, periodical) stimulation of anaerobic, mesophilic, methane-producing processes, performed by electric square impulses, is a major feature of the present invention. Stimulation is performed in two stages of the sewage treatment process; or it can be performed in the first stage if the acetogenic-methanogenic, mostly methanogenic micropopulation of the inoculum is adapted to a sewage having a concentration of 0.1 - 3.5 g COD/l which is eventually diluted. This cyclic stimulation is performed, - depending on the origin and composition of the sewage - for 1 - 20 days, for the second time when the COD concentration of the sewage is beginning to get increased. Naturally, when no dilution of the sewage is necessary no second stimu-

lation cycle is required either. The second period of cyclic stimulation is usually performed for 5 - 40 days, in the meantime the granulate bed, consisting of 1 - 5 mm particles of the anaerobic, mesophilic, mixed micropopulation with high, mostly methanogenic activity, is developed.

According to a further embodiment of the present invention instead of the granulate a biofilm with high methanogenic activity, immobilized on a carrier, is formed. Any type of zeolite, preferably Akvarosorb^{R} is used as carrier.

According to the second process variant 10 - 50 % (v/v), preferably 25 - 35 % (v/v), of the inoculum, if desired, pretreated by electric stimulation, is filled up to 100 % (v/v) with sewage (concentration 1 - 4 g COD/l), the system is homogenized then its pH is adjusted to 6.5 - 7.5. The system containing both the inoculum and the sewage is then cultivated under anaerobic conditions in the mesophilic temperature range for 5 - 10, preferably 6 - 8 days, then 50 - 80 % (v/v), preferably 65 - 70 % (v/v) of the fermentation broth is removed and the system is filled up with sewage of the same volume and COD concentration, thereafter fermentation is continued as described above for the same time. After concluded second fermentation 0.1 - 3.0 % (w/v) of a carrier (particle size 0.2 - 0.3 mm) is added to the fermentation broth [usually some zeolite type mineral is applied as carrier, one of them, Akvarosorb^{R} (National Ore and Mineral Mines, Hegyalja, Mád, Hungary) proved to be the carrier of choice] and the system is stirred for a couple of days (1 - 4 days). After concluded stirring the suspension is left to settle, the supernatant, which amounts usually to 80 - 90 % (v/v) of the total volume, depending on the amount of carrier added to the system, is decanted. The retained biomass, immobilized on the

carrier, is transferred into a fermentor, described at the first variant of the process of the invention, where vigorous ascending recirculation is maintained for a couple of days, thereafter purification is pursued according to the former process variant.

Regulation of exact pH values is of utmost importance throughout the entire operation. Usually operations should be pursued in the pH range of 6 - 8, preferably at pH 6.8 - 7.5.

The following examples are illustrating but not limiting the scope of the invention.

Example 1

Preparation of inoculum and increasing the digesting activity of anaerobic, digested sewage sludge

Operation is performed in 1500 ml glass jar fermentors equipped with an air-tight cover made of insulating material. Two stainless steel electrodes (diameter 2 mm), placed to a distance of 85 mm from each other, are immersed vertically into the fermentor through the cover. The length of the electrodes should be chosen to enable an immersion of 130 mm in the sludge.

a.) 1000 ml of freshly digested sewage sludge, removed from the 2000 cu.m. digester of the municipal sewage treatment plant, is filled into the glass fermentor which is closed, connected to a gasometer and placed into an air-conditioned room of 33 - 35°C. The sludge is homogenized once daily for 5 minutes, then 100 ml of the sludge is removed and replaced by 100 ml of digested sewage sludge then the system is again homogenized for 5 minutes. This semicontinuous (daily removal and addition) digestion is

continued for 7 days to attain a new equilibrium and the operation (removal-addition) is repeated daily.

b.) On the 8th day - following the removal-addition cycles described above - the system is submitted to electric stimulation by a generator for 8 hours daily.

Parameters of electric stimulation:

Type	Square impulse
Amplitude	80 V
Frequency	20 Hz
Refractory time	15 sec
Duration of stimulation per day	8 hours followed by a 16 hour interval

The results of the digestion performed for 7 days without stimulation then for the subsequent 10 days under electric stimulation are presented in Table 1.

Table 1

	No electric stimulation	Electric stimulation	Change %
Digested sewage sludge:			
dissolved COD (g/l)	2.8	1.6	-28.6
biogas formation (ml/l)	435	762	+75.2
biogas composition % (v/v):			
CH_4	62	70	+12.9
CO_2	38	30	-21.1
generated CH_4 (ml/l)	269.7	533.4	+97.7

Data of the table demonstrate that upon electric stimulation

a.) the dissolved COD of the sludge is significantly reduced (-28.5 %),

b.) biogas formation, a typical feature of methanogenic activity, is significantly increased (+75.2 %),

c.) the amount of the methane formed is nearly doubled compared to the unstimulated sample (this increases the heating value of the biogas which represents the basis for the rentability of the operation), finally,

d.) the ballast material content of the biogas (CO_2) is significantly reduced.

Example 2

Continuous sewage treatment (municipal)

a.) The operation is performed in a 2000 ml

glass jar fermentor equipped with two vertical stainless steel electrodes (diameter 2 mm).

1500 ml of freshly digested sewage sludge, removed from the anaerobic digester of a municipal sewage treatment plant, is transferred to a fermentor, placed in an air-conditioned room of 33 - 36°C and is continuously recirculated in an ascending flow with a pump for 2 days.

b.) On the third day the recirculation of the digested sewage sludge is stopped and the continuous influx of the sewage is started: 1500 ml/day of the municipal sewage (0.5 g/l COD) is led into the fermentor at the bottom and an equal amount of the overflow is collected in a container. Simultaneously the electrode-outlets are connected to a generator and electric stimulation is started.

Parameters of electric stimulation:

Type	Square impulse
Amplitude	80 V
Frequency	20 Hz
Refractory time	10 sec
Duration of stimulation per day	12 hours followed by a 12 hour interval

Thereafter the mixed acetogenic-methanogenic micropopulation and the COD concentration of the continuous sewage input are gradually increased to 14.0 g/l and the system is adapted to the conversion of the high organic matter content (COD) to methane by applying the above described electric stimulation during 12 hours. The electric stimulation is continued for 15 days. Upon the gradual increase of COD loading and electric stimulation, from the 15th - 20th day of adaptation microorganism-aggregates are formed and by the 30th - 35th day of adaptation the formation of

granulates with high methanogenic activity (diameter 2 - 4 mm) in the fermentor is concluded.

Parameters on the 35th day of adaptation:

COD concentration of sewage influx in the fermentor	14 g/l
COD concentration of sewage efflux in the fermentor	0.56 g/l
Output of biogas conversion	96 %
Biogas production per fermentor volume	5.8 l/l fermentor/day
Methane content of biogas	80 %

Technical parameters of sewage treatment performed in the same fermentor with the same digested sewage sludge at identical conditions, with and without electric stimulation as described in Example 2, are presented in Table 2.

Table 2

	No electric stimulation	Electric stimulation	Change %
Adaptation time (day)	80	35	-56.25
COD of effluent sewage (g/l)	1.54	0.56	-63.6
COD conversion (%)	89	96	+ 7
Biogas generated (l/l fermentor/day)	5.2	5.8	+11.5
Composition of biogas:			
CH_4 %	74	80	+ 6
CO_2 %	26	20	- 6

Data of the table demonstrate that upon electric stimulation

a.) the adaptation time and

b.) the COD concentration of effluent sewage are significantly reduced while

c.) the amount of generated biogas and

d.) the energy content of the biogas (CH_4) are increased.

Example 3

Treatment of industrial sewage containing volatile fatty acids

a.) Three litres of digested sewage sludge submitted to electric stimulation according to the procedure described in Example 1, b.) are transferred into a fermentor with a working volume of 10 l, then 7 l of sewage (1.5 g/l of COD) containing lower alkyl (C_2 - C_5) fatty acids is added, the mixture is stirred and its pH is adjusted with a sodium hydroxide solution to 6.8 - 7.2. The fermentor is closed, stored in an air-conditioned room at 33 - 35°C and submitted to batch fermentation at anaerobic conditions for 7 days.

On the 7th day of the batch fermentation inoculation is carried out, after vigorous stirring 7 l of the fermentation broth is removed, discarded and to the residual 3 l of inoculum remaining in the fermentor, a new portion of 7 l of sewage (1.5 g/l of COD) is added, the pH is adjusted with sodium hydroxide solution to 6.8 - 7.2, the fermentor is closed and anaerobic batch fermentation is carried out for a further 7 days.

On the 6th - 8th day after inoculation 50 g of "Akvarosorb®" carrier powder (particle size 0.2 - 0.3

mm) (National Ore and Mineral Mines, Hegyalja, Mád, Hungary) are added and the mixture is slowly stirred for 48 hours then 8.5 l of the clear supernatant is decanted and discarded.

b.) The residual biomass, immobilized on 1500 ml of the carrier, is transferred into a glass jar fermentor and is continuously recirculated in ascending flow for 2 days.

On the third day recirculation is stopped and the operation is continued as described in Example 2, paragraph b.) except that the adaptation is initiated with sewage containing 1.5 g/l of COD and 1.2 g/l of volatile fatty acid which is continuously led through the biomass immobilized on the carrier.

Parameters of the electric stimulation applied:

Type	Square impulse
Amplitude	100 V
Frequency	40 Hz
Refractory time	30 sec
Duration of stimulation per day	8 hours followed by a 16 hour interval

Thereafter the COD concentration of the sewage containing fatty acids is continuously and gradually increased to 30 g/l and the system is submitted to adaptation by electric stimulation during 8 hours daily to promote the conversion of the high organic matter content (COD, volatile fatty acids) to methane. Electric stimulation is pursued during the first 10 days of adaptation.

From the 20th day of adaptation sewage of 30 g/l COD is continuously fed into the fermentor.

The parameters of the sewage treatment process equilibrium are the following:

COD concentration of sewage influx in the

fermentor 30 g/l
COD concentration of sewage efflux in the fermentor 2.4 g/l
Output of biogas conversion 92 %
Biogas production per fermentor volume 7.5 l/l fermentor/day
Methane content of biogas 78 %

If sewage treatment is carried out in the same fermentor at identical conditions, without electric stimulation a sewage of maximum 18 g/l of COD can be treated at a conversion rate of 85 % and adaptation time of 50 days.

The results are presented in Table 3.

Table 3

	No electric stimulation	Electric stimulation	Change %
Adaptation time (day)	50	20	-60
COD of sewage influx (g/l)	18	30	+66.6
COD of effluent sewage (g/l)	2.7	2.4	-11
COD conversion (%)	85	92	+ 7
Biogas generated (l/l fermentor/day)	4.3	7.5	+74
Composition of biogas:			
CH_4 %	71	78	+ 7
CO_2 %	29	22	- 7

Example 4

Treatment of dairy sewage

a.) The procedure specified in Example 3, paragraph a.) is applied except that 7 l of dairy sewage (3 g/l COD) is added to 3 l of electrically stimulated inoculum. Thereafter inoculation, batch fermentation, mixing with the carrier (Akvarosorb[R]) and recirculation are pursued according to Example 3, paragraphs a.) and b.).

After concluded recirculation the procedure described in Example 3, paragraph b.) is follwed except that adaptation is performed with the continuous feeding of dairy sewage (3 g/l COD) for 8 days under fluid conditions and electric stimulation with the following parameters:

Type	Square impulse
Amplitude	80 V
Frequency	20 Hz
Refractory time	15 sec
Duration of stimulation per day	5 hours followed by a 19 hour interval

Equilibrium parameters on the 12th day of adaptation:

COD concentration of sewage influx in the fermentor	3 g/l
COD concentration of sewage efflux in the fermentor	0.09 g/l
Output of biogas conversion	97 %
Biogas production per fermentor volume	1.3 l/l fermentor/day
Methane content of biogas	75 %

Example 5

Process for improving the B12 coenzyme production of fermentation broths

Operation is performed in a 2500 ml glass jar fermentor equipped with an air-tight cover made of insulating material. Two stainless steel electrodes are immersed at a distance of 100 mm from one another vertically into the fermentor (diameter 3 mm). The length of the electrodes ensures 170 mm immersion in the fermentation broth.

a.) 2000 ml of a broth resulting from a B12 coenzyme producing, semicontinuous fermentation carried out in a glass jar fermentor according to the process specified in the Hungarian Patent Specification No. 188,955 (equivalent U. S. Patent Specification No. 4,659,661) is submitted to semiautomatic fermentation for 5 days according to the process described in Example 1, paragraph b.). The active ingredient content of the broth (B12 coenzyme + factor III) is assayed daily according to Hungarian Patent Specification No. 188,955, Example 2, paragraph a.) column 2, section 6, together with the rate of methanolysis. The active ingredient content of the broth amounts to 17.5 mg of B12 coenzyme/l fermentation broth, i. e. the daily active ingredient production amounts to 1.75 mg of B12 coenzyme/l fermentation broth at a daily broth removal of 10 %. The daily methanol consumption required for this level of active ingredient yield is 4.8 g/l broth. The rate of methanolysis is 0.2 g methanol/l fermentation broth/hour. The amount of methanol required for the production of 1 mg/l broth/day of active ingredient is 2.74 g /l broth/day.

b.) From the 6th day of the operation fermentation is continued as formerly (removing 10 % of

the broth and adding a medium specified in the Hungarian Patent Specification No. 188,955, page 6, column 1) and the electric stimulation of the fermentation is started by means of the two electrodes and the generator mounted on the fermentor.

Parameters of the electric stimulation:

Type	Square impulse
Amplitude	150 V
Frequency	30 Hz
Refractory time	20 sec
Duration of stimulation per day	2 x 5 hours 2 x 7 hours interval.

Thereafter fermentation is continued unchanged according to the process specified in the Hungarian Patent Specification No. 188,955, Example 1, paragraph b.) and the cyclic electric stimulation is repeated as described above. In the course of a 5-day fermentation period the rate of methanolysis is increased to 0.37 g methanol/l fermentation broth/hour, i. e. the daily added 4.8 g/l of methanol is utilized by the microorganisms within 13 hours. The active ingredient production remains unchanged, 1.75 mg/l fermentation broth/day.

c.) Thereafter both fermentation and stimulation are performed according to the process specified above (paragraph b.) except that the original amount of daily added methanol - 4.8 g methanol/l fermentation broth/day - is increased proportionally to the rate of methanolysis, to 8.8 g/l fermentation broth/day of methanol and the fermentation is continued for additional 10 days. During the 10-day fermentation period the active ingredient content of the fermentation broth is increased to 32 mg B12 coenzyme/l fermentation broth, resulting in an increased production capacity which is proportional to the increase of specific methanol utilization.

Results of the fermentation are presented in

Table 4.

Table 4

	No electric stimulation	Electric stimulation	Change %
Specific B12 coenzyme production (mg/l broth/day)	1.75	3.2	+83
Rate of methanolysis (g methanol/l broth/hour)	0.20	0.37	+85
Specific methanol utilization (g methanol/mg B12 coenzyme/day	2.75	2.75	0

What we claim is

1. A process for intensification of fermentations, which comprises applying electric stimulation of the fermentation medium in any stage or stages of the fermentation.

2. A process as claimed in claim 1, which comprises applying the electric stimulation in the inoculum preparing stage and/or in the adaptation stage and/or in the stacioner (producing) stage of the fermentation.

3. A process as claimed in claim 1, which comprises applying the electric stimulation in the inoculum preparing stage of the fermentation.

4. A process as claimed in claim 1, which comprises applying the electric stimulation in the adaptation stage of the fermentation.

5. A process as claimed in claim 1, which comprises applying the electric stimulation in the inoculum preparing and adaptation stage of the fermentation.

6. A process as claimed in claim 1, which comprises applying the electric stimulation in the inoculum preparing, adaptation and stacioner (producing) stage of the fermentation.

7. A process as claimed in any of claims 1 to 6, which comprises applying the electric stimulation in an anaerobic, mesophilic, methane-producing fermentation.

8. A process as claimed in any of claims 1 to 6, which comprises applying the electric stimulation in an anaerobic, mesophilic, methane-producing fermentation for improving preferably sewage treatment procedures pursued under septic conditions by acetogenic-methanogenic, mostly methanogenic micropopulations.

9. A process as claimed in claim 3, which comprises

a) 25 - 35 % (v/v) of an inoculum and 65 -

75 % (v/v) of a 1 - 4 g COD/litre sewage (calculated for working volume) are added to a batch fermentor, the system is homogenized, and the pH is adjusted to 6.5 - 7.5, then

b) batch fermentation is pursued in the mesophilic temperature range, under anaerobic conditions for 5 - 10, preferably for 6 - 8 days, then,

c) after 5 - 10, preferably 6 - 8 days, and following homogenization, 65 - 75 % (v/v) of the fermentation broth is removed and an equal volume of sewage, having a concentration of 1 - 4 g COD/litre, is added and the batch fermentation is repeated according to the procedure specified in paragraph a2.),

d) the batch fermentation is followed for further 5 - 12, preferably 6 - 10 day among similar conditions but the fermentation broth is electrically stimulated,

e) the obtained electrically stimulated inoculum is collected.

10. A process as claimed in claim 8, which comprises

a) loading an air-tight fermentor, equipped with two electrodes, with a sewage sludge freshly obtained from the the final digester of a municipal sewage treatment plant, which is preferably pretreated with cyclic electric stimulation, and maintaining an ascending, recirculating flow in the mesophilic temperature range for 1 - 4 days, then

b) adding daily at least equal volumes of 0.1 - 3.5 g COD/litre sewage to the inoculum formed, starting cyclic electric stimulation, maintaining an intense ascending flow of the system, transferring the biogas formed to a gasometer and collecting the overflow which has equal volume with that of the sewage influx, thereafter

c) the COD loading of the sewage to be treated is gradually increased to a concentration of 3 -

40 g COD/litre, simultaneously the cyclic electric stimulation is maintained for 5 - 30 days, during which period a biomass of high methanogenic activity, adapted to the particular sewage, is developed, thereafter the electric stimulation is stopped and the sewage treatment is continued, if desired,

d) the treated sewage is discarded or purified further, the biogas formed is transferred into a gasometer and utilized.

11. A process as claimed in any of claims 1 to 10, which comprises applying a mesophilic temperature range of 30 - 40°C, preferably 31 - 33°C.

12. A process as claimed in any of claims 1 to 11, which comprises using an electric stimulation induced by direct current square impulses at 30 - 250 V, 5 - 50 Hz frequency and 5 - 50 sec refractory time.

13. A process as claimed in any of claims 1 to 12, which comprises using an electric stimulation induced by direct current square impulses at 80 - 150 V, 20 - 40 Hz frequency and 10 - 30 sec refractory time.

14. A process as claimed in any of claims 1 to 13, which comprises applying cyclic electric stimulation for 5 - 12 hours followed by an interval of 7 - 19 hours.

15. A process as claimed in any of claims 1 to 14, which comprises applying a B12 coenzyme micropopulation in the fermentation.

16. A process as claimed in claim 15, which comprises carrying out the electric stimulation by direct current square impulses, having an amplitude of 100 - 220 V, a frequency of 20 - 50 Hz and a refractory time of 10 - 40 sec.

17. A process as claimed in claims 15, which comprises carrying out electric stimulation by direct current square impulses, having an amplitude of 120 - 180 V, preferably 140 - 160 V, a frequency of 25 - 40 Hz and

a refractory time of 15 - 30 sec.

18. A process as claimed in any of claims 15 to 17, which comprises inducing the stimulation for 4 - 6 hours followed by an interval of 6 - 8 hours.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/HU 93/00059

A. CLASSIFICATION OF SUBJECT MATTER

IPC^5: C 02 F 3/28, 11/04, 1/48; C 12 N 13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC^5: C 02 F 3/28, 11/04, 1/48, 3/34; C 12 N 13/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Austrian Patents

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Questel WPI(L)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/ AT	Authorized officer
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/HU 93/00059

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